

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/16014

## A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl<sup>7</sup> C12N9/88, 15/09, 1/15, 1/19, 1/21, 5/10, C12P13/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl<sup>7</sup> C12N9/88, 15/09, 1/15, 1/19, 1/21, 5/10, C12P13/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubMed, BIOSIS/WPI (DIALOG), JSTPlus (JOIS)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 9-275978 A (Mitsui Toatsu Chemicals, Inc.), 28 October, 1997 (28.10.97), (Family: none)	1-6, 10-17, 23-34, 41-43, 45-55

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search  
11 March, 2004 (11.03.04)Date of mailing of the international search report  
23 March, 2004 (23.03.04)Name and mailing address of the ISA/  
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

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## Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
(See extra sheet.)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
(See extra sheet.)

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

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Continuation of Box No. II of continuation of first sheet(1)

## •Nitrile hydratase per se

First, the "nitrile hydratases having an  $\alpha$ -subunit and a  $\beta$ -subunit" as set forth in claims 1 to 12 will be discussed. There are presented 5 $\alpha$ -subunit types (i.e., the unsubstituted one, one having substitution at the 36-position, one having substitution at the 71-position, - - - and one having substitution at the 204-position) and 15  $\beta$ -subunit types (i.e., the unsubstituted one, one having substitution at the 10-position, one having substitution at the 32-position, - - - and one having substitution at the 217-position). That is, there are presented at least 75 types (5x15) of nitrile hydratases.

To conclude that these 75 nitrile hydratases comply with the requirement of unity of invention, they should be so linked as to form a single general inventive concept. In other words, these combinations should have a technical relationship involving the same "special technical features". The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over "the prior art". (See, if required, PCT Rule 13.2.)

However, "JP 9-275978 A (Mitsui Toatsu Chemicals, Inc.) 1997.10.28" (hereinafter referred to as the referential document) having been publicly known before the priority date of the present case reports a plural number of "nitrile hydratases having an  $\alpha$ -subunit and a  $\beta$ -subunit" having amino acid substitutions at different positions from the above-described ones and yet substantially sustaining the nitrile hydratase function. Thus, it cannot be referred to as a "special technical feature" to merely substantially sustain the nitrile hydratase function after substitution. The fact that these 75 nitrile hydratases are obtained by a modification method not described in the referential document (for example, the modification method as set forth in claim 56) cannot be regarded as "a special technical feature", unless it can be concluded that these "substances", i.e., the 75 nitrile hydratases cannot be obtained any other method than the above modification method. Even though the statement in the description of the present case is taken into consideration, the presence of any other "special technical feature" (for example, such a technical feature that "all" of these 75 nitrile hydratases sustain higher activity than the nitrile hydratase reported in the referential document) can be neither confirmed nor designated.

Such being the case, the above-described 75 nitrile hydratases cannot be considered as a group of inventions so linked as to form a single general inventive concept.

The same applies to the genes, plasmids, etc. as set forth in claims 13 to 55, because of relating to the above-described 75 nitrile hydratases.

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Continuation of Box No. II of continuation of first sheet(1)

## •Method of modifying enzyme having nitrile hydratase activity

First, claims 56 to 71 will be discussed. Modification of nitrile hydratase per se is reported in the referential document too. In other words, a modification method has been already reported. Therefore, the modification methods as set forth in claims 56 to 71 should have a technical relationship involving the same "special technical feature" exceeding a mere modification method.

However, the invention as set forth in claim 56 and the invention as set forth in claim 57, for example, differ in sequential data, etc. to be referred in discussing the modification. The same applies to claims 58 to 71 too. Even though the statement in the description of the present case is taken into consideration, the presence of any other "special technical feature" can be neither confirmed nor designated.

Such being the case, it is considered that claims 56 to 71 have inventions relating to 16 types of modification methods as set forth in claims 56 to 71, even though dependent claims are taken into consideration.

The same applies to the modified enzymes, etc. as set forth in claims 72 to 77 because of being specified as obtained by the 16 modification methods as described above.

## •Conclusion

Even though the 75 nitrile hydratases are obtained by modification methods that are not described in the referential document (i.e., the 16 inventions relating to the methods of modifying an enzyme having nitrile hydratase activity), there is observed no "special technical feature" between the nitrile hydratases per se and the methods of modifying an enzyme having nitrile hydratase activity, unless it can be concluded that these "substances", i.e., the 75 nitrile hydratases cannot be obtained any other method than the above modification method.

Thus, it is concluded that the present case has 91 inventions in total including the 75 inventions relating to nitrile hydratases and the 16 inventions relating to methods of modifying an enzyme having nitrile hydratase activity.

Since no required additional search fees were timely paid by the applicant, the international search report is made exclusively on the part of the invention according to claim 1 relating to a nitrile hydratase in which the  $\alpha$ -subunit "has an amino acid sequence wherein the amino acid at the 36-position in SEQ ID NO:1 is substituted by another amino acid" and the parts of the inventions according to claims 2 to 6, 10 to 17, 23 to 34, 41 to 43 and 45 to 55 in which the  $\alpha$ -subunit "has an amino acid sequence wherein the amino acid at the 36-position in SEQ ID NO:1 is substituted by another amino acid" or a base sequence corresponding thereto.